

ANTI-HPV T CELL RECEPTORS AND ENGINEERED CELLS

CLAIM OF PRIORITY

This application claims the benefit of international Application No.

- 5 PCT/CN2020/074366, filed on February 5, 2020. The entire contents of the foregoing application are incorporated herein by reference.

TECHNICAL FIELD

- 10 The present disclosure relates to T cells receptors that recognize or bind to a cancer antigen, engineered cells, and cell-based therapies.

BACKGROUND

- Cancer is one of the most widespread cellular anomalies caused by biological and environmental factors, such as age, gender, genetic mutations, environmental exposure such as
15 UV radiation, occupational risk factors, carcinogens, asbestos, radioactive materials, and viral infections (e.g., HPV, EBV, HBV, HCV, HTLV-1 and KSHV) (Margaret E *et al.*, “Viruses Associated With Human Cancer,” *Biochimica et Biophysica Acta*.1782:127–150 (2008)). Particularly, some cancers (e.g., cervical cancer) are primarily caused by virus (e.g., human papilloma virus, HPV) infection (Stanley *et al.*, “HPV: From Infection To Cancer.” *Biochemical*
20 *Society Transactions*: 35: part 6 (2007)).

Despite advancement in treatments such as chemotherapy, the efficacy of various treatments for cancers, including HPV associated cancers, is relatively poor. Accordingly, there exists an unmet need for effective therapies for cancers.

- 25 **SUMMARY**

The present disclosure is related to T cells receptors that recognize or bind tumor antigen human papilloma virus (HPV) E6, genetically engineered cells, and cell therapies for treating HPV associated cancers.

- 30 In one example, the disclosure is related to a T cell receptor (TCR) or antigen-binding fragment thereof, comprising an alpha chain comprising a variable alpha (Va) region and a beta chain comprising a variable beta (Vb) region. In some examples, the Va region comprises a

complementarity determining region 1 (CDR-1), a complementarity determining region 2 (CDR-2), and a complementarity determining region 3 (CDR-3); in some examples, the Va region CDR-1 comprises an amino acid sequence that is identical to a selected Va region CDR-1 amino acid sequence, the Va region CDR-2 comprises an amino acid sequence that is identical to a
5 selected Va region CDR-2 amino acid sequence, and the Va region CDR-3 comprises an amino acid sequence that is identical to a selected Va region CDR-3 amino acid sequence; and in some examples, the Vb region comprises a complementarity determining region 1 (CDR-1), a complementarity determining region 2 (CDR-2), and a complementarity determining region 3 (CDR-3); in some examples, the Vb region CDR-1 comprises an amino acid sequence that is
10 identical to a selected Vb region CDR-1 amino acid sequence, the Vb region CDR-2 comprises an amino acid sequence that is identical to a selected Vb region CDR-2 amino acid sequence, and the Vb region CDR-3 comprises an amino acid sequence that is identical to a selected Vb region CDR-3 amino acid sequence. In some examples, the selected Va region CDR-1, CDR-2, and CDR-3 amino acid sequences and the selected Vb region CDR-1, CDR-2, and CDR-3 amino
15 acid sequences are one of the following:

- (1) the selected Va region CDR-1, CDR-2, and CDR-3 amino acid sequences are set forth in SEQ ID NOs: 5, 6, and 7, respectively, and the selected Vb region CDR-1, CDR-2, and CDR-3 amino acid sequences are set forth in SEQ ID NOs: 8, 9, and 10, respectively;
- (2) the selected Va region CDR-1, CDR-2, and CDR-3 amino acid sequences are set forth in
20 SEQ ID NOs: 27, 28, and 29, respectively, and the selected Vb region CDR-1, CDR-2, and CDR-3 amino acid sequences are set forth in SEQ ID NOs: 30, 31, and 32, respectively;
- (3) the selected Va region CDR-1, CDR-2, and CDR-3 amino acid sequences are set forth in SEQ ID NOs: 33, 34, and 35, respectively, and the selected Vb region CDR-1, CDR-2, and CDR-3 amino acid sequences are set forth in SEQ ID NOs: 36, 37, and 38, respectively;
- (4) the selected Va region CDR-1, CDR-2, and CDR-3 amino acid sequences are set forth in
25 SEQ ID NOs: 39, 40, and 41, respectively, and the selected Vb region CDR-1, CDR-2, and CDR-3 amino acid sequences are set forth in SEQ ID NOs: 42, 43, and 44, respectively.

In one aspect, the present disclosure refers to a T cell receptor (TCR) or antigen-binding fragment thereof, comprising an alpha chain comprising a variable alpha (Va) region and a beta
30 chain comprising a variable beta (Vb) region, wherein:

the Va region comprises a complementarity determining region 1 (CDR-1), a complementarity determining region 2 (CDR-2), and a complementarity determining region 3 (CDR-3), wherein the Va region CDR-1 comprises an amino acid sequence that is identical to a selected Va region CDR-1 amino acid sequence, the Va region CDR-2 comprises an amino acid sequence that is identical to a selected Va region CDR-2 amino acid sequence, and the Va region CDR-3 comprises an amino acid sequence that is identical to a selected Va region CDR-3 amino acid sequence; and

the Vb region comprises a complementarity determining region 1 (CDR-1), a complementarity determining region 2 (CDR-2), and a complementarity determining region 3 (CDR-3), wherein the Vb region CDR-1 comprises an amino acid sequence that is identical to a selected Vb region CDR-1 amino acid sequence, the Vb region CDR-2 comprises an amino acid sequence that is identical to a selected Vb region CDR-2 amino acid sequence, and the Vb region CDR-3 comprises an amino acid sequence that is identical to a selected Vb region CDR-3 amino acid sequence;

wherein the selected Va region CDR-1, CDR-2, and CDR-3 amino acid sequences and the selected Vb region CDR-1, CDR-2, and CDR-3 amino acid sequences are one of the following:

(1) the selected Va region CDR-1, CDR-2, and CDR-3 amino acid sequences are set forth in SEQ ID NOs: 5, 6, and 7, respectively, and the selected Vb region CDR-1, CDR-2, and CDR-3 amino acid sequences are set forth in SEQ ID NOs: 8, 9, and 10, respectively;

(2) the selected Va region CDR-1, CDR-2, and CDR-3 amino acid sequences are set forth in SEQ ID NOs: 27, 28, and 29, respectively, and the selected Vb region CDR-1, CDR-2, and CDR-3 amino acid sequences are set forth in SEQ ID NOs: 30, 31, and 32, respectively;

(3) the selected Va region CDR-1, CDR-2, and CDR-3 amino acid sequences are set forth in SEQ ID NOs: 33, 34, and 35, respectively, and the selected Vb region CDR-1, CDR-2, and CDR-3 amino acid sequences are set forth in SEQ ID NOs: 36, 37, and 38, respectively; and

(4) the selected Va region CDR-1, CDR-2, and CDR-3 amino acid sequences are set forth in SEQ ID NOs: 39, 40, and 41, respectively, and the selected Vb region CDR-1, CDR-2, and CDR-3 amino acid sequences are set forth in SEQ ID NOs: 42, 43, and 44, respectively.

In some examples, the Va region comprises CDR-1, CDR-2, and CDR-3 with the amino acid sequences set forth in SEQ ID NOs: 5, 6, and 7, respectively; and the Vb region comprises CDR-1, CDR-2, and CDR-3 with the amino acid sequences set forth in SEQ ID NOs: 8, 9, and 10, respectively.

In some examples, the Va region comprises the amino acid sequence set forth in any of SEQ ID NO: 1, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto; and the Vb region comprises the amino acid sequence set forth in any of SEQ ID NO: 2, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

In some examples, the Va region comprises CDR-1, CDR-2, and CDR-3 with the amino acid sequences set forth in SEQ ID NOs: 27, 28, and 29, respectively; and the Vb region comprises CDR-1, CDR-2, and CDR-3 with the amino acid sequences set forth in SEQ ID NOs: 30, 31, and 32, respectively.

In some examples, the Va region comprises the amino acid sequence set forth in any of SEQ ID NO: 45, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto; and the Vb region comprises the amino acid sequence set forth in any of SEQ ID NO: 46 or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

In some examples, the Va region comprises CDR-1, CDR-2, and CDR-3 with the amino acid sequences set forth in SEQ ID NOs: 33, 34 and 35, respectively; and the Vb region comprises CDR-1, CDR-2, and CDR-3 with the amino acid sequences set forth in SEQ ID NOs: 36, 37, and 38, respectively.

In some examples, the Va region comprises the amino acid sequence set forth in any of SEQ ID NO: 47, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto; and the Vb region comprises the amino acid sequence set forth in any of SEQ ID NO: 48 or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

In some examples, the Va region comprises CDR-1, CDR-2, and CDR-3 with the amino acid sequences set forth in SEQ ID NOs: 39, 40, and 41, respectively; and the Vb region comprises CDR-1, CDR-2, and CDR-3 with the amino acid sequences set forth in SEQ ID NOs: 42, 43, and 44, respectively.

In some examples, the Va region comprises the amino acid sequence set forth in any

of SEQ ID NO: 49, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto; and the Vb region comprises the amino acid sequence set forth in any of SEQ ID NO: 50 or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

In some examples, the alpha chain comprises a mouse alpha chain constant region, and the beta chain comprises a mouse beta chain constant region.

In some examples, the alpha chain comprises the amino acid sequence set forth in any of SEQ ID NO: 15, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto; and the beta chain comprises the amino acid sequence set forth in any of SEQ ID NO: 16, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

In some examples, the alpha chain comprises the amino acid sequence set forth in any of SEQ ID NO: 51, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto; and the beta chain comprises the amino acid sequence set forth in any of SEQ ID NO: 52, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

In some examples, the alpha chain comprises the amino acid sequence set forth in any of SEQ ID NO: 53, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto; and the beta chain comprises the amino acid sequence set forth in any of SEQ ID NO: 54, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

In some examples, the alpha chain comprises the amino acid sequence set forth in any of SEQ ID NO: 55, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto; and the beta chain comprises the amino acid sequence set forth in any of SEQ ID NO: 56, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

In some examples, the TCR or antigen-binding fragment thereof binds to or recognizes a peptide epitope of E6 (SEQ ID NO: 19) that is presented by a major histocompatibility complex (MHC) molecule.

In some examples, the MHC molecule is an HLA-A2 molecule.

5 In some examples, the TCR or antigen-binding fragment thereof, when expressed on the surface of a T cell, stimulates cytotoxic activity against a target cancer cell.

In some examples, the target cancer cell comprises HPV DNA sequences or expresses E6.

10 In one example, the disclosure is related to a T cell receptor (TCR) or antigen-binding fragment thereof, comprising an alpha chain comprising a variable alpha (Va) region and a beta chain comprising a variable beta (Vb) region. In some examples, the Va region comprises a complementarity determining region 1 (CDR1), a complementarity determining region 2 (CDR2), and a complementarity determining region 3 (CDR3), and the Vb region comprises a CDR1, a CDR2, and a CDR3; in some examples,

15 (1) the Va region CDR1, CDR2, and CDR3 are identical to complementarity determining regions 1, 2, and 3 in SEQ ID NO: 1, respectively, and the Vb region CDR1, CDR2, and CDR3 are identical to complementarity determining regions 1, 2, and 3 in SEQ ID NO: 2, respectively;

(2) the Va region CDR1, CDR2, and CDR3 are identical to complementarity determining regions 1, 2, and 3 in SEQ ID NO: 45, respectively, and the Vb region CDR1, CDR2, and CDR3
20 are identical to complementarity determining regions 1, 2, and 3 in SEQ ID NO: 46, respectively;

(3) the Va region CDR1, CDR2, and CDR3 are identical to complementarity determining regions 1, 2, and 3 in SEQ ID NO: 47, respectively, and the Vb region CDR1, CDR2, and CDR3 are identical to complementarity determining regions 1, 2, and 3 in SEQ ID NO: 48, respectively;
or

25 (4) the Va region CDR1, CDR2, and CDR3 are identical to complementarity determining regions 1, 2, and 3 in SEQ ID NO: 49, respectively, and the Vb region CDR1, CDR2, and CDR3 are identical to complementarity determining regions 1, 2, and 3 in SEQ ID NO: 50, respectively.

In one aspect, the present disclosure refers to a T cell receptor (TCR) or antigen-binding fragment thereof, comprising an alpha chain comprising a variable alpha (Va) region and a beta
30 chain comprising a variable beta (Vb) region, wherein:

the Va region comprises a complementarity determining region 1 (CDR1), a complementarity determining region 2 (CDR2), and a complementarity determining region 3 (CDR3), and the Vb region comprises a CDR1, a CDR2, and a CDR3, wherein

(1) the Va region CDR1, CDR2, and CDR3 are identical to complementarity determining regions 1, 2, and 3 in SEQ ID NO: 1, respectively, and the Vb region CDR1, CDR2, and CDR3 are identical to complementarity determining regions 1, 2, and 3 in SEQ ID NO: 2, respectively;

(2) the Va region CDR1, CDR2, and CDR3 are identical to complementarity determining regions 1, 2, and 3 in SEQ ID NO: 45, respectively, and the Vb region CDR1, CDR2, and CDR3 are identical to complementarity determining regions 1, 2, and 3 in SEQ ID NO: 46, respectively;

(3) the Va region CDR1, CDR2, and CDR3 are identical to complementarity determining regions 1, 2, and 3 in SEQ ID NO: 47, respectively, and the Vb region CDR1, CDR2, and CDR3 are identical to complementarity determining regions 1, 2, and 3 in SEQ ID NO: 48, respectively; or

(4) the Va region CDR1, CDR2, and CDR3 are identical to complementarity determining regions 1, 2, and 3 in SEQ ID NO: 49, respectively, and the Vb region CDR1, CDR2, and CDR3 are identical to complementarity determining regions 1, 2, and 3 in SEQ ID NO: 50, respectively.

In one example, the disclosure is related to a vector comprising a nucleic acid encoding the TCR or antigen-binding fragment thereof as described herein. In one aspect, the present disclosure refers to a vector comprising a nucleic acid encoding any one of the TCR or antigen-binding fragment thereof as described herein.

In some examples, the vector is an expression vector, a viral vector, a retroviral vector, or a lentiviral vector.

In one example, the disclosure is related to a vector including: a) a first nucleic acid sequence encoding a TCR alpha chain comprising an alpha chain variable region of a human anti-E6 TCR and an alpha chain constant region; and b) a second nucleic acid sequence encoding a TCR beta chain comprising a beta chain variable region of the human anti-E6 TCR and a beta chain constant region. In one aspect, the present disclosure refers to a vector comprising: a) a first nucleic acid sequence encoding a TCR alpha chain comprising an alpha chain variable region of a human anti-E6 TCR and an alpha chain constant region; and b) a second nucleic acid sequence encoding a TCR beta chain comprising a beta chain variable region of the human anti-E6 TCR and a beta chain constant region.

In some examples, the alpha chain constant region is a human TCR alpha chain constant region and the beta chain constant region is a human TCR beta chain constant region.

In some examples, the alpha chain constant region is a mouse TCR alpha chain constant region and the beta chain constant region is a mouse TCR beta chain constant region.

In some examples, the first nucleic acid sequence and the second nucleic acid sequence are linked by a linker sequence. In some examples, the linker sequence is a P2A sequence.

In some examples, the first nucleic acid sequence comprises a sequence set forth in SEQ ID NO: 17, or a nucleic acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto; and the second nucleic acid sequence comprises a sequence set forth in SEQ ID NO: 18, or a nucleic acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In some examples, the first nucleic acid sequence comprises a sequence set forth in SEQ ID NO: 57, or a nucleic acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto; and the second nucleic acid sequence comprises a sequence set forth in SEQ ID NO: 58, or a nucleic acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto; In some examples, the first nucleic acid sequence comprises a sequence set forth in SEQ ID NO: 59, or a nucleic acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto; and the second nucleic acid sequence comprises a sequence set forth in SEQ ID NO: 60, or a nucleic acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In some examples, the first nucleic acid sequence comprises a sequence set forth in SEQ ID NO: 61, or a nucleic acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto; and the second nucleic acid sequence comprises a sequence set forth in SEQ ID NO: 62, or a nucleic acid sequence that has

at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

5 In some examples, the vector as described herein further includes a third nucleic acid sequence encoding a checkpoint inhibitor.

In some examples, the checkpoint inhibitor is an antibody.

In some examples, the checkpoint inhibitor is an anti-PD-1 antibody scFv, or an anti-CTLA4 antibody scFv.

10 In some examples, the antibody comprises a heavy chain variable domain comprising an amino acid sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 11; and a light chain variable domain comprising an amino acid sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 12.

15 In some examples, the third nucleic acid sequence comprises a nucleic acid sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 13; and a nucleic acid sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 14.

In some examples, the vector is an expression vector, a viral vector, a retroviral vector, or a lentiviral vector. In some examples, the retroviral vector is pMP71.

20 In some examples, the vector comprises (1) a nucleic acid sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 20; (2) a nucleic acid sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 63; (3) a nucleic acid sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 64; or
25 (4) a nucleic acid sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 65.

In one example, the disclosure is related to an engineered cell comprising the vector as described herein.

30 In one example, the disclosure is related to an engineered cell, comprising the TCR or antigen-binding fragment thereof as described herein. In one aspect, the present disclosure refers to an engineered cell, comprising any one of the TCR or antigen-binding fragment thereof as described herein.

In some examples, the TCR or antigen binding fragment thereof is heterologous to the cell.

In some examples, the engineered cell is a cell line.

In some examples, the engineered cell is a primary cell obtained from a subject (e.g., a human subject).

5 In some examples, the engineered cell is a T cell. In some examples, the T-cell is isolated from a human subject. In some examples, the T cell is CD8+. In some examples, the T cell is CD4+.

In one example, the disclosure is related to a method for producing the engineered cell, comprising introducing the vector as described herein into a cell *in vitro* or *ex vivo*.

10 In some examples, the vector is a viral vector and the introducing is carried out by transduction.

In one example, the disclosure is related to a method of treating a disease or a disorder, comprising administering the engineered cell as described herein to a subject having a disease or disorder associated with HPV. In one aspect, the present disclosure refers to use of the engineered cell described herein in the manufacture of a medicament for treating a disease or a disorder in a subject having a disease or disorder associated with HPV.

In some examples, the disease or disorder associated with HPV is a cancer. In some examples, the cancer is a cancer of the head and neck, uterine cervix, oropharynx, anus, anal canal, anorectum, vagina, vulva, or penis.

20 In one example, the disclosure is related to a method of treating a tumor in a subject, the method comprising administering to the subject in need thereof (a) an engineered T cell, comprising: a nucleic acid encoding a TCR or antigen-binding fragment thereof that specifically binds to an HPV antigen; and (b) a checkpoint inhibitor.

In some examples, the tumor is an HPV-induced tumor.

25 In one example, the disclosure is related to a TCR or antigen-binding fragment thereof that cross competes with the TCR or antigen-binding fragment thereof as described herein.

In one example, the disclosure provides a method of administering to a patient an effective amount of genetically engineered anti-cancer human T cells to treat a disease, disorder or condition in the patient, wherein the genetically engineered anti-cancer human T cells express an anti-tumor T-cell receptor for HPV E6 antigen. In some examples, the alpha chain of an anti-tumor T-cell receptor is encoded by the nucleotide sequence of SEQ ID NO: 3, and the beta chain is encoded by the nucleotide sequence of SEQ ID NO: 4. In some examples, the alpha chain of an anti-tumor T-cell receptor is encoded by the nucleotide sequence of SEQ ID NO: 78, and the beta chain is encoded by the nucleotide sequence of SEQ ID NO: 79. In some

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examples, the alpha chain of an anti-tumor T-cell receptor is encoded by the nucleotide sequence of SEQ ID NO: 80, and the beta chain is encoded by the nucleotide sequence of SEQ ID NO: 81. In some examples, the alpha chain of an anti-tumor T-cell receptor is encoded by the nucleotide sequence of SEQ ID NO: 82, and the beta chain is encoded by the nucleotide sequence of SEQ ID NO: 83. In some examples, the alpha chain of the anti-tumor T cell receptor has a variable alpha ($V\alpha$) region comprising an amino acid sequence of SEQ ID NO: 1 and beta chain of the anti-tumor human T cell receptor has a variable beta ($V\beta$) region comprising an amino acid sequence of SEQ ID NO: 2. In some examples, the alpha chain of the anti-tumor T cell receptor has a variable alpha ($V\alpha$) region comprising an amino acid sequence of SEQ ID NO: 45, and beta chain of the anti-tumor human T cell receptor has a variable beta ($V\beta$) region comprising an amino acid sequence of SEQ ID NO: 46. In some examples, the alpha chain of the anti-tumor T cell receptor has a variable alpha ($V\alpha$) region comprising an amino acid sequence of SEQ ID NO: 47, and beta chain of the anti-tumor human T cell receptor has a variable beta ($V\beta$) region comprising an amino acid sequence of SEQ ID NO: 48. In some examples, the alpha chain of the anti-tumor T cell receptor has a variable alpha ($V\alpha$) region comprising an amino acid sequence of SEQ ID NO: 49, and beta chain of the anti-tumor human T cell receptor has a variable beta ($V\beta$) region comprising an amino acid sequence of SEQ ID NO: 50. The disease, disorder or condition can be cancer-related, such as cervical cancer, head and neck cancer, oropharyngeal cancers, anal cancer, penile cancer, vaginal cancer and vulvar cancer.

In one example, the disclosure also provides a T cell receptor. In some instances, the alpha chain of the anti-tumor human T cell receptor has a sequence of a variable alpha ($V\alpha$) region (SEQ ID NO: 1) and the beta chain of the anti-tumor human T cell receptor has a sequence of variable beta ($V\beta$) region (SEQ ID NO: 2). In some instances, the alpha chain of the anti-tumor human T cell receptor has a sequence of a variable alpha ($V\alpha$) region (SEQ ID NO: 45) and the beta chain of the anti-tumor human T cell receptor has a sequence of variable beta ($V\beta$) region (SEQ ID NO: 46). In some instances, the alpha chain of the anti-tumor human T cell receptor has a sequence of a variable alpha ($V\alpha$) region (SEQ ID NO: 47) and the beta chain of the anti-tumor human T cell receptor has a sequence of variable beta ($V\beta$) region (SEQ ID NO: 48). In some instances, the alpha chain of the anti-tumor human T cell receptor has a sequence of a variable alpha ($V\alpha$) region (SEQ ID NO: 49) and the beta chain of the anti-tumor human T cell receptor

has a sequence of variable beta ($V\beta$) region (SEQ ID NO: 50). In some instances, the variable alpha ($V\alpha$) region of the anti-tumor human T cell receptor is fused to a constant region of a mouse T-cell receptor alpha chain. In some instances, the variable beta ($V\beta$) region of the anti-tumor human T cell receptor is fused to a constant region of a mouse T-cell receptor beta chain.

5 In one example, the disclosure provides an engineered T cell comprising a nucleic acid encoding a genetically engineered antigen receptor that specifically binds to human papilloma virus (HPV) antigen E6.

In one example, the disclosure further provides a method for patient-specific T-cell therapy, wherein a gene is engineered into patient-specific T cells and delivered back into the
10 patient as a therapeutic agent.

The present disclosure further provides a method of diagnosing a disease/condition, wherein the condition can include cancer, and wherein the disease can be diagnosed by analyzing the complex formed as a result of the contact between the T-cell receptors with the sample from the patient/mammal to be diagnosed, and wherein the complex can be detected by any of the
15 means well-known in the art. In some examples, the results can be used to determine whether the cell therapy will be effective.

The present disclosure further provides a pharmaceutical composition comprising an engineered T cell receptor (TCR) or an antigen-binding fragment thereof having antigenic specificity for human papillomavirus (HPV) antigen E6 and a pharmaceutically acceptable
20 carrier.

The present disclosure also provides a vector system for transfecting cells with a chimeric gene, wherein the vector system includes nucleic acid sequences encoding the variable region of the alpha chain of a human anti-E6 TCR, nucleic acid sequences encoding the variable region of the beta chain of same human anti-E6 TCR and a linker sequence.

25 As used herein, the term “about” refers to a measurable value such as an amount, a time duration, and the like, and encompasses variations of $\pm 20\%$, $\pm 10\%$, $\pm 5\%$, $\pm 1\%$, $\pm 0.5\%$ or $\pm 0.1\%$ from the specified value.

As used herein, the term “HPV antigen” refers to a polypeptide molecule derived from human papilloma virus (HPV). In some examples, the HPV is HPV1, HPV2, HPV3, HPV4,
30 HPV6, HPV10, HPV11, HPV16, HPV18, HPV26, HPV27, HPV28, HPV29, HPV30, HPV31, HPV33, HPV34, HPV35, HPV39, HPV40, HPV41, HPV42, HPV43, HPV45, HPV49, HPV51,

HPV52, HPV54, HPV55, HPV56, HPV57, HPV58, HPV59, HPV68, or HPV69. Particularly, the HPV can be a high risk HPV, for example, HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV68, or HPV69. In some examples, the HPV polypeptide molecule is selected from E6.

5 As used herein, the term "peripheral blood cells" refers to cells normally found in the peripheral blood including, but is not limited to, eosinophils, neutrophils, T cells, monocytes, K cells, granulocytes, and B cells.

 As used herein, the term "genetically engineered cell" or "genetically modified cell" refers to a cell with a modification of a nucleic acid sequence in the cell, including, but not
10 limited to, a cell having a insertion, deletion, substitution, or modification of one or more nucleotides in its genome, and a cell with an exogenous nucleic acid sequence (e.g., a vector), wherein the exogenous nucleic acid sequence is not necessarily integrated into the genome.

 As used herein, the term "cancer" or "cancer cell" refers to the cells dividing in an uncontrolled manner. Examples of such cells include cells having an abnormal state or condition
15 characterized by rapidly proliferating cell growth. The term is meant to include cancerous growths, e.g., tumors; oncogenic processes, metastatic tissues, and malignantly transformed cells, tissues, or organs, irrespective of histopathologic type or stage of invasiveness. The cancer cells can form the solid tumors or the excessive tumor cells in blood (e.g., hematologic cancer). Alternatively or additionally it can include all types of cancerous growths or oncogenic
20 processes, metastatic tissues or malignantly transformed cells, tissues, or organs, irrespective of histopathologic type or stage of invasiveness. Examples of solid tumors include malignancies, e.g., sarcomas, adenocarcinomas, and carcinomas, of the various organ systems, such as those affecting liver, lung, breast, lymphoid, gastrointestinal (e.g., colon), genitourinary tract (e.g., renal, urothelial cells), prostate and pharynx. Adenocarcinomas include malignancies such as
25 most colon cancers, rectal cancer, renal-cell carcinoma, liver cancer, non-small cell carcinoma of the lung, cancer of the small intestine and cancer of the esophagus. Examples of cancers that can be treated by the methods described herein include e.g., bone cancer, pancreatic cancer, skin cancer (e.g., melanoma), cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach
30 cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva,

Hodgkin Disease, non-Hodgkin lymphoma, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemias including acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, and/or T-cell lymphoma.

As used herein, the term “HPV associated cancer” refers to cancers that are associated or caused by HPV infection.

As used herein, the term “vector” refers to a vehicle by which a polynucleotide sequence (e.g. a foreign gene) can be introduced into a host cell, in order to obtain the desired gene expression of the introduced nucleotide sequence. Cloning vectors can include e.g., plasmids, phages, viruses, etc. Most popular type of vector is a "plasmid", which refers to a closed circular double stranded DNA loop into which additional DNA segments comprising gene of interest can be ligated. Another type of vector is a viral vector, in which a nucleic acid construct to be transported is ligated into the viral genome. Viral vectors are capable of autonomous replication in a host cell into which they are introduced or may integrate themselves into the genome of a host cell and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" or simply "expression vectors". In some examples, the vectors are viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses).

As used herein, a "subject" is a mammal, such as a human or a non-human animal. In some examples, the subject, e.g., patient, to whom the cells, cell populations, or compositions are administered is a mammal, typically a primate, such as a human. In some examples, the primate is a monkey or an ape. The subject can be male or female and can be any suitable age, including infant, juvenile, adolescent, adult, and geriatric subjects. In some examples, the subject is a non-primate mammal, such as a dog, a cat, a horse, a rodent, a rat, or a mouse.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Methods and materials are described herein for use in the present disclosure; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

Other features and advantages of the disclosure will be apparent from the following detailed description and figures, and from the claims.

DESCRIPTION OF THE DRAWINGS

Exemplary embodiments are illustrated in referenced figures. It is intended that the embodiments and figures disclosed herein are to be considered illustrative rather than restrictive.

FIG. 1 is a schematic diagram showing a pMP71 retroviral vector construct. P2A encodes a 2A self-cleaving peptide; Va encodes the variable region of the alpha chain of a human anti-E6 TCR; Vb encodes the variable region of the beta chain of the same human anti-E6 TCR; Ca encodes the constant region of the mouse TCR alpha chain; Cb encodes the constant region of the mouse TCR beta chain. Ψ indicates packaging sequences on viral RNA. 5'LTR and 3'LTR are long terminal repeats.

FIG. 2A shows the expression of TCR in non-transduced human primary T cells. NT is a non-transduced control. After 48 hours of culture, expression of the recombinant TCR was detected by staining mouse TCR beta chain. A viable CD3⁺ lymphocyte gating strategy was used.

FIG. 2B shows the expression of E202 TCR in human primary T cells transduced with the E202 construct. After 48 hours of culture, expression of the recombinant TCR was detected by staining mouse TCR beta chain. A viable CD3⁺ lymphocyte gating strategy was used.

FIG. 3A is a graph showing the intracellular IFN- γ expression of non-transduced human T cells upon antigen-specific stimulation. NT is a non-transduced control. The non-transduced human T cells were co-cultured overnight with target cells expressing the HPV E6 antigen at 1:1 effector-to-target ratio. The T cells were then collected and the intracellular IFN- γ expression was determined by flow cytometry.

WHAT IS CLAIMED IS:

1. A T cell receptor (TCR) or antigen-binding fragment thereof, comprising an alpha chain comprising a variable alpha (Va) region and a beta chain comprising a variable beta (Vb) region, wherein:

5 the Va region comprises a complementarity determining region 1 (CDR-1), a complementarity determining region 2 (CDR-2), and a complementarity determining region 3 (CDR-3), wherein the Va region CDR-1 comprises an amino acid sequence that is identical to a selected Va region CDR-1 amino acid sequence, the Va region CDR-2 comprises an amino acid sequence that is identical to a selected Va region CDR-2 amino acid sequence, and the Va region
10 CDR-3 comprises an amino acid sequence that is identical to a selected Va region CDR-3 amino acid sequence; and

the Vb region comprises a complementarity determining region 1 (CDR-1), a complementarity determining region 2 (CDR-2), and a complementarity determining region 3 (CDR-3), wherein the Vb region CDR-1 comprises an amino acid sequence that is identical to a
15 selected Vb region CDR-1 amino acid sequence, the Vb region CDR-2 comprises an amino acid sequence that is identical to a selected Vb region CDR-2 amino acid sequence, and the Vb region CDR-3 comprises an amino acid sequence that is identical to a selected Vb region CDR-3 amino acid sequence;

wherein the selected Va region CDR-1, CDR-2, and CDR-3 amino acid sequences and the
20 selected Vb region CDR-1, CDR-2, and CDR-3 amino acid sequences are one of the following:

(1) the selected Va region CDR-1, CDR-2, and CDR-3 amino acid sequences are set forth in SEQ ID NOs: 5, 6, and 7, respectively, and the selected Vb region CDR-1, CDR-2, and CDR-3 amino acid sequences are set forth in SEQ ID NOs: 8, 9, and 10, respectively;

(2) the selected Va region CDR-1, CDR-2, and CDR-3 amino acid sequences are set forth in
25 SEQ ID NOs: 27, 28, and 29, respectively, and the selected Vb region CDR-1, CDR-2, and CDR-3 amino acid sequences are set forth in SEQ ID NOs: 30, 31, and 32, respectively;

(3) the selected Va region CDR-1, CDR-2, and CDR-3 amino acid sequences are set forth in SEQ ID NOs: 33, 34, and 35, respectively, and the selected Vb region CDR-1, CDR-2, and CDR-3 amino acid sequences are set forth in SEQ ID NOs: 36, 37, and 38, respectively; and

30 (4) the selected Va region CDR-1, CDR-2, and CDR-3 amino acid sequences are set forth in SEQ ID NOs: 39, 40, and 41, respectively, and the selected Vb region CDR-1, CDR-2, and

CDR-3 amino acid sequences are set forth in SEQ ID NOs: 42, 43, and 44, respectively.

2. The TCR or antigen-binding fragment thereof of claim 1, wherein the Va region comprises CDR-1, CDR-2, and CDR-3 with the amino acid sequences set forth in SEQ ID NOs: 5, 6, and 7, respectively; and the Vb region comprises CDR-1, CDR-2, and CDR-3 with the amino acid sequences set forth in SEQ ID NOs: 8, 9, and 10, respectively.
3. The TCR or antigen-binding fragment thereof of claim 2, wherein:
 - the Va region comprises an amino acid sequence that has at least 80% sequence identity to SEQ ID NO: 1; and
 - the Vb region comprises an amino acid sequence that has at least 80% sequence identity to SEQ ID NO: 2.
4. The TCR or antigen-binding fragment thereof of claim 1, wherein the Va region comprises CDR-1, CDR-2, and CDR-3 with the amino acid sequences set forth in SEQ ID NOs: 27, 28, and 29, respectively; and the Vb region comprises CDR-1, CDR-2, and CDR-3 with the amino acid sequences set forth in SEQ ID NOs: 30, 31, and 32, respectively.
5. The TCR or antigen-binding fragment thereof of claim 4, wherein:
 - the Va region comprises an amino acid sequence that has at least 80% sequence identity to SEQ ID NO: 45; and
 - the Vb region comprises an amino acid sequence that has at least 80% sequence identity to SEQ ID NO: 46.
6. The TCR or antigen-binding fragment thereof of claim 1, wherein the Va region comprises CDR-1, CDR-2, and CDR-3 with the amino acid sequences set forth in SEQ ID NOs: 33, 34 and 35, respectively; and the Vb region comprises CDR-1, CDR-2, and CDR-3 with the amino acid sequences set forth in SEQ ID NOs: 36, 37, and 38, respectively.
7. The TCR or antigen-binding fragment thereof of claim 6, wherein:
 - the Va region comprises an amino acid sequence that has at least 80% sequence identity

to SEQ ID NO: 47; and

the Vb region comprises an amino acid sequence that has at least 80% sequence identity to SEQ ID NO: 48.

- 5 8. The TCR or antigen-binding fragment thereof of claim 1, wherein the Va region comprises CDR-1, CDR-2, and CDR-3 with the amino acid sequences set forth in SEQ ID NOs: 39, 40, and 41, respectively; and the Vb region comprises CDR-1, CDR-2, and CDR-3 with the amino acid sequences set forth in SEQ ID NOs: 42, 43, and 44, respectively.

- 10 9. The TCR or antigen-binding fragment thereof of claim 8, wherein:
the Va region comprises an amino acid sequence that has at least 80% sequence identity to SEQ ID NO: 49; and
the Vb region comprises an amino acid sequence that has at least 80% sequence identity to SEQ ID NO: 50.

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10. The TCR or antigen-binding fragment thereof of any one of claims 1-9, wherein:
the alpha chain comprises a mouse alpha chain constant region, and the beta chain comprises a mouse beta chain constant region.

- 20 11. The TCR or antigen-binding fragment thereof of any of claims 1-10, wherein the TCR or antigen-binding fragment thereof binds to or recognizes a peptide epitope of E6 (SEQ ID NO: 19) that is presented by a major histocompatibility complex (MHC) molecule.

- 25 12. The TCR or antigen-binding fragment thereof of claim 11, wherein the MHC molecule is an HLA-A2 molecule.

13. A T cell receptor (TCR) or antigen-binding fragment thereof, comprising an alpha chain comprising a variable alpha (Va) region and a beta chain comprising a variable beta (Vb) region, wherein:

- 30 the Va region comprises a complementarity determining region 1 (CDR1), a complementarity determining region 2 (CDR2), and a complementarity determining region 3

(CDR3), and the Vb region comprises a CDR1, a CDR2, and a CDR3, wherein

(1) the Va region CDR1, CDR2, and CDR3 are identical to complementarity determining regions 1, 2, and 3 in SEQ ID NO: 1, respectively, and the Vb region CDR1, CDR2, and CDR3 are identical to complementarity determining regions 1, 2, and 3 in SEQ ID NO: 2, respectively;

5 (2) the Va region CDR1, CDR2, and CDR3 are identical to complementarity determining regions 1, 2, and 3 in SEQ ID NO: 45, respectively, and the Vb region CDR1, CDR2, and CDR3 are identical to complementarity determining regions 1, 2, and 3 in SEQ ID NO: 46, respectively;

(3) the Va region CDR1, CDR2, and CDR3 are identical to complementarity determining regions 1, 2, and 3 in SEQ ID NO: 47, respectively, and the Vb region CDR1, CDR2, and CDR3 are identical to complementarity determining regions 1, 2, and 3 in SEQ ID NO: 48, respectively;
10 or

(4) the Va region CDR1, CDR2, and CDR3 are identical to complementarity determining regions 1, 2, and 3 in SEQ ID NO: 49, respectively, and the Vb region CDR1, CDR2, and CDR3 are identical to complementarity determining regions 1, 2, and 3 in SEQ ID NO: 50, respectively.

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14. A vector comprising a nucleic acid encoding the TCR or antigen-binding fragment thereof of any of claims 1-13.

15. A vector comprising:

- 20 a) a first nucleic acid sequence encoding a TCR alpha chain comprising an alpha chain variable region of a human anti-E6 TCR and an alpha chain constant region; and
b) a second nucleic acid sequence encoding a TCR beta chain comprising a beta chain variable region of the human anti-E6 TCR and a beta chain constant region.

25 16. The vector of claim 15, wherein the alpha chain constant region is a mouse TCR alpha chain constant region and the beta chain constant region is a mouse TCR beta chain constant region.

17. The vector of any one of claims 15-16, further comprising a third nucleic acid sequence
30 encoding a checkpoint inhibitor.

18. The vector of claim 17, wherein the checkpoint inhibitor is an anti-PD-1 antibody scFv, or an anti-CTLA4 antibody scFv.

5 19. An engineered cell, comprising the TCR or antigen-binding fragment thereof of any of claims 1-13.

20. Use of the engineered cell of claim 19 in the manufacture of a medicament for treating a disease or a disorder in a subject having a disease or disorder associated with HPV.

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